Supplementary Information

Molecular chaperone prefoldin-assisted biosynthesis of gold nanoparticles with improved size distribution and dispersion

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Experimental

1. Preparation of glycerol dehydrogenase (GLD) and prefoldin (PFD)

Glycerol dehydrogenase enzyme (GLD) was expressed and purified as described[1]. In brief, *Escherichia coli* bearing pET22-His-tag-GLD plasmid was pre-cultured until the culture optical density (OD) reached 0.50, and the expression of the GLD proteins was subsequently induced *via* the addition of isopropyl *β*-D-thiogalactoside at final concentration 0.5 mM. GLD was purified with Ni-NTA column, and desalted using PD-10 column with buffer Exchange to 20 mM Tris-HCl (pH 7.4). GLD concentration was measured using a BCA assay and the purity of enzyme was determined using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis (Fig. S2).

Prefoldin (PFD) was expressed in *E. coli* and purified as described[2]. In brief, *E. coli* BL21 (DE3) cells bearing expression plasmids were cultured in Lysogeny broth (LB) medium containing 100 μg/ml ampicillin. The cells were disrupted by sonication on ice. The supernatant after centrifugation was subjected to heat treatment at 70°C for 30 min, and denatured proteins were removed by centrifugation. The supernatant was applied to a cation exchange column (CM-TOYOPEAL, TOSOH, Tokyo, Japan) and a gel-filtration column (HiLoad 26/60, GE Healthcare, Little Chalfont, UK) for purification. The purified PFD was dialyzed with 10kDa dialysis membrane cut-off in PBS buffer (pH 7.4) as dialysate. PFD concentration was measured using a bicinchoninic acid (BCA) assay and purity of enzyme was determined using SDS-PAGE analysis (Fig. S2).

2. Synthesis of AuNP using GLD in the presence of PFD

AuNPs was synthesized using GLD as previously described[3]. In brief, for the preparation of 100 μL sample, 10 μL of 25 mM Au ion ($HAuCl_4$) (f. 2.5 mM) was mixed with 5.6 μL of 17.8 μM GLD (f. 1 μM), 10 μL of 4 mM NAD^+ (f. 400 μM) and 10 μL of 1 M glycerol (f. 100 mM) in 50 mM Glycine-NaOH buffer (pH 9.40) in the absence or presence of PFD, and incubated at 37°C for 48 h. AuNPs was synthesized in the presence of 200 nM BSA as a control experiment. The samples were stored at 4°C before use.

3. Characterization

Dynamic Light Scattering (DLS)

Prior to DLS measurement, contaminants (e.g. dust, foreign particles, other proteins and large aggregates) were eliminated by centrifugation (7220 rpm (5000 *g)*, 30 sec, 4^{o}C) to ensure the measurement quality. Nanoparticle size was estimated with DLS Intensity measurement using

Zetasizer Nano-ZS (Malvern, Worcestershire, UK). The integration time of the scattered light for each DLS measurement was 30 sec with three repeated measurements per setup.

Darkfield microscopy (DFM)

DFM observation of the AuNP samples was carried out using a BX53 microscope (Olympus, Tokyo, Japan) equipped with a UP73 CCD camera, UPlanFLN 60× objective lens, and U-DCW dark field condenser as described [4]. The AuNP samples synthesized in the absence or presence of 200 nM PFD diluted 20 times with MilliQ water (Merck Millipore, Burlington, USA) were dropped on 3-Aminopropyltriethoxysilane (APTES)-treated slide glass, and covered with a cover glass. Each spot in the DFM images was separated to three images of Red (R), Green (G) and Blue (B) components and the intensity of each component image was obtained to using ImageJ software. The greenness of each spot was calculated using the following equation;

Greeness = G – (R+B)/2

, where R, G, B indicate the intensity of each RGB component [5]. The ratio of spots whose greenness >17 are counted as "green" spots, which were considered to be the monomer. The average values from three independent images were shown.

Zeta potential

The zeta potentials of the AuNP samples were determined using a Malvern Nano ZS90 system at 25 °C. The AuNP samples synthesized in the absence or presence of 200 nM PFD diluted 10 times with MilliQ water were applied into the cell (DTS1060C-Cleatdisposable zeta cell). Zeta potentials were calculated from the electrophoretic mobility using a Smoluchowki relationship. The average values of three independent measurements were shown.

Fourier transform infrared spectroscopy (FTIR)

Attenuated total reflectance fourier transform infrared (ATR-FTIR) spectra were recorded on a Nicolet iS5 FTIR spectrometer equipped with iD5 ATR accessory (Thermo Fisher Scientific, Waltham, USA) from 1750 to 1450 cm^{-1} with an accumulation of 32 scans. The AuNP synthesized in the presence of 200 nM PFD (200 μL) was centrifuged (9000 rpm, 15 min), and the supernatant was removed. The sample was then washed with 200 μL of D_2O twice, and re-dispersed in 5 μL of D_2O. GLD and PFD solutions (10 μM each) were used for comparison. Two μL of the aliquots from the samples were spread on the surface of a diamond crystal, and air-dried prior to the measurement.

Transmission electron microscopy (TEM) and field-emission scanning electron microscope with energy dispersive X-ray spectrometer (SEM-EDX)

TEM measurements were performed with JEM-2100 transmission electron microscope (JEOL, Tokyo, Japan) operated at 80 or 120 kV. Aliquots from the AuNP sample were dropped on a carbon-coated copper grid, and air-dried. FE-SEM-EDX (JEOL JSM-7100FA) was used for observation of AuNP and for compositional analysis. The samples were dropped on a silicon surface, and air-dried prior to the measurement.

4. Evaluation of the protein bound to AuNPs

Silver staining was conducted for characterization of proteins bound to AuNPs. To remove unbound proteins and concentrate the solutions, the protein-AuNP solutions were washed and centrifuged using Glycine-NaOH buffer (pH 9.5) at 150,000 rpm on 4°C three times. Samples were resuspended in SDS 2×sample buffer [0.25 M Tris-HCl (pH 6.8), 4 wt% SDS, 20 wt% glycerol, 0.01 wt% bromophenol blue, and 12 wt% 2-mercaptoethanol] and heated at 94 °C for 15 min to detach and denature proteins. Samples (10 μL) were loaded on a 12.5% SDS-polyacrylamide gel for electrophoresis on 150 V voltage, 20 mA current for 80 minutes.

For silver staining, gels were treated with Silver Stain II Kit (Wako, Osaka, Japan). The intensities of appearing bands are quantified using BioRad (Hercules, USA) software to make standard curves for determining final concentration of both GLD and PFD attached to AuNPs (Fig. S3). The amount of bound proteins (GLD and PFD) was calculated using the standard curves shown in the figure.

References

1. Y. Hosomomi, T. Niide, R. Wakabayashi, M. Goto and N. Kamiya, *Anal Sci*, 2016, **32**, 295.
2. T. Zako, Y. Murase, R. Iizuka, T. Yoshida, T. Kanzaki, N. Ide, M. Maeda, T. Funatsu and M. Yohda, *J Mol Biol*, 2006, **364**, 110.
3. T. Niide, M. Goto and N. Kamiya, *Chem Commun*, 2011, **47**, 7350.
4. T. Bu, T. Zako, M. Fujita and M. Maeda, *Chem Commun*, 2013, **49**, 7531.
5. K. Sato, M. Onoguchi, Y. Sato, K. Hosokawa and M. Maeda, *Anal Biochem*, 2006, **350**, 162.

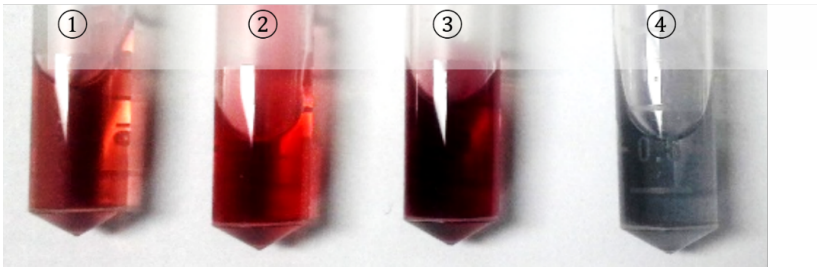

Figure S1 AuNPs synthesized using enzymatic reaction of purified GLD with different concentration of $HAuCl_{4(aq)}$ addition incubated at 37°C, 200 rpm for 48 hours. From left to right:① 0.25 mM $HAuCl_{4(aq)}$, ② 0.75 mM $HAuCl_{4(aq)}$, ③1.50 mM $HAuCl_{4(aq)}$, ④ 2.50 mM $HAuCl_{4(aq}$

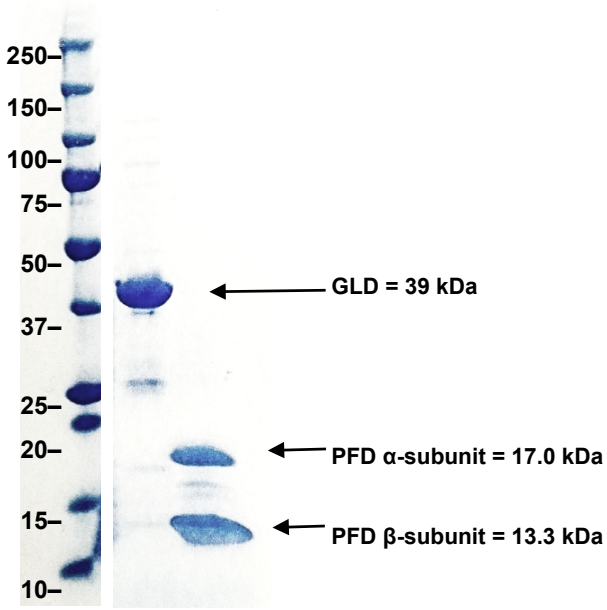

Figure S*2* SDS-PAGE (12.5% polyacrylamide) analysis of the purified GLD and PFD.

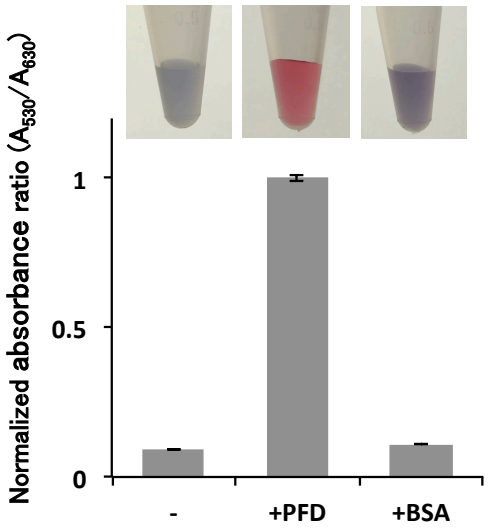

Figure S3 AuNPs synthesized in the presence of BSA. Photographs and the normalized ratio of absorbance at 530 nm / 630 nm of AuNPs formed using GLD in the absence (-) and presence of PFD (+PFD) or BSA (+BSA) were shown.

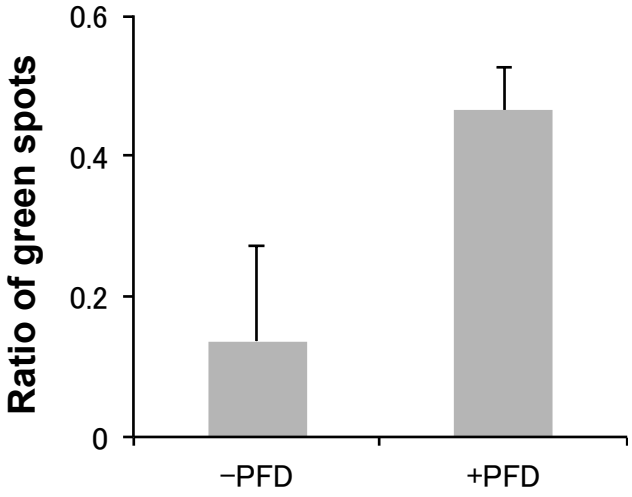

Figure S4 Ratio of green spots in the DFM images shown in Fig.2d.

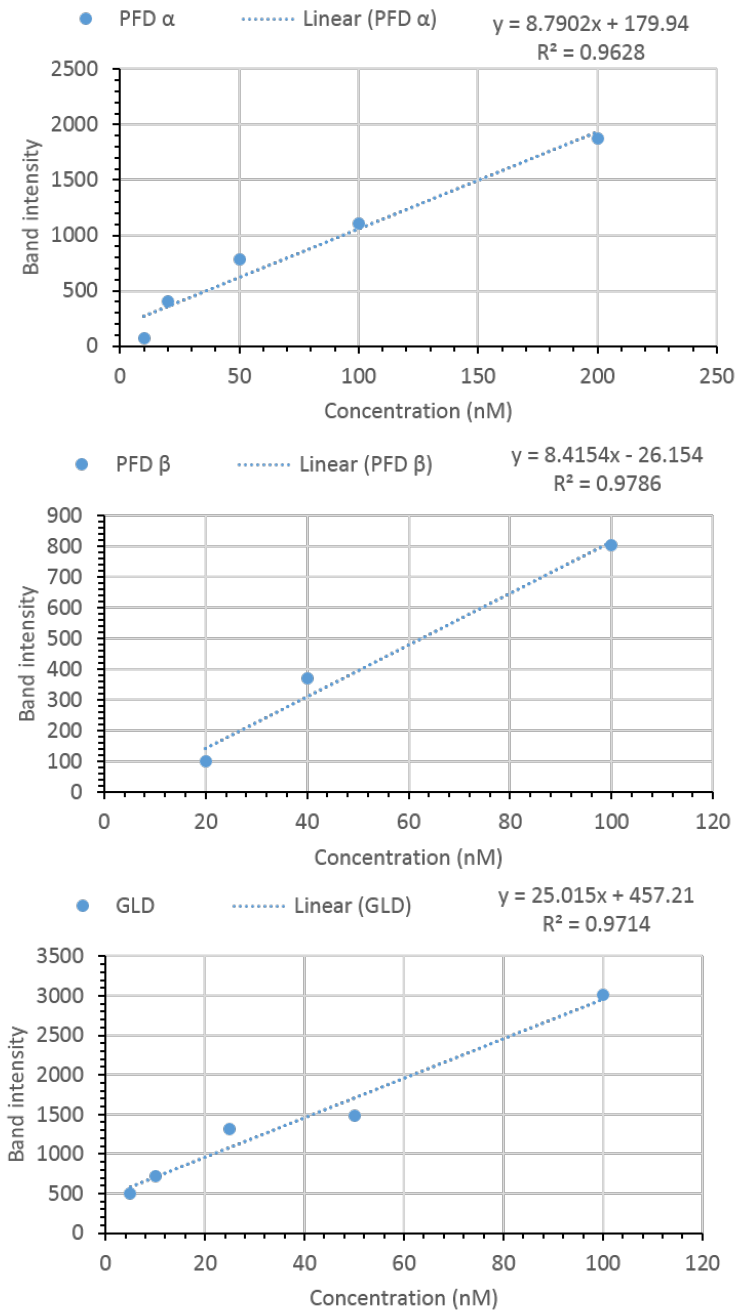

Figure S5 Standard curves of a) PFD α-subunit and b) β-subunit and c) GLD from SDS-PAGE/silver staining assay (Fig. 3).

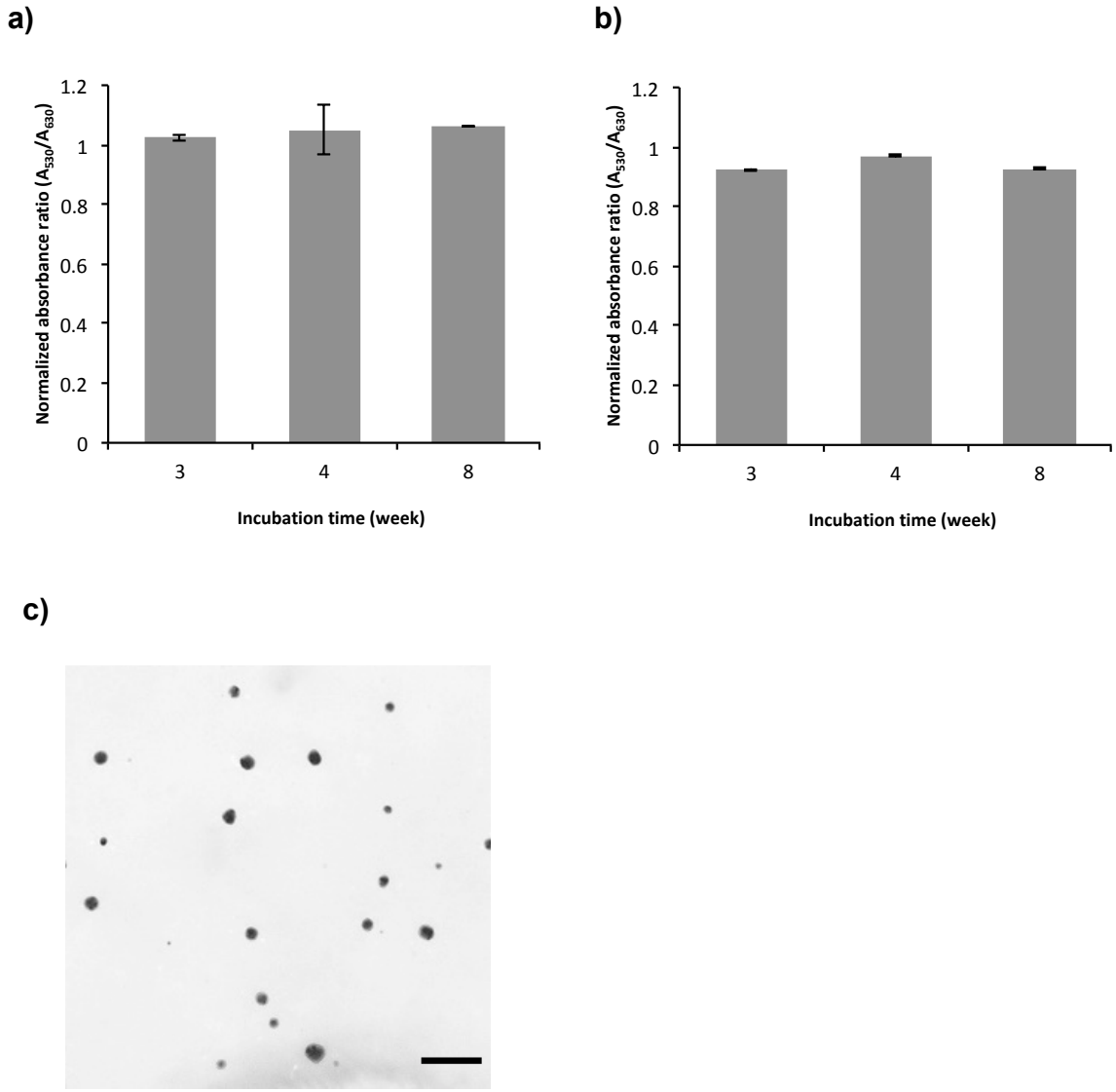

Figure S6 Stability of AuNPs synthesized in the presence of 200 nM PFD. Following the AuNPs synthesis, absorbance of each sample after incubation for a certain time period was measured. Absorbance ratios at 530 and 630 nm after incubation at a) room temperature or b) 4^{o}C for 3, 4 and 8 weeks were shown. The ratio before incubation was normalized as 1.0. The average values of three independent measurements were shown. (c) TEM image of AuNPs synthesized in the presence of PFD after 1 week storage at 4 ^{o}C. Scale bar=100 nm